An Evaluation of Some Commercial Test Papers and Tablets for the Determination of Glucose in Urine

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ABSTRACT

The commercial test papers, Tes-Tape, Clinistix, Uristix and Combistix, and the tablet preparation, Clinitest, were evaluated as indicators of glucose in urine by means of a quantitative automated glucose oxidase procedure for glucose determination. The semiquantitative Tes-Tape yielded very low values on urine specimens when compared with the quantitative method. More reliable results could be obtained with this product if the urine specimens were first treated with a mixed bed resin to remove inhibitors of the glucose oxidase peroxidase system. The qualitative test papers, Clinistix, Uristix and Combistix, yielded responses in closer agreement with the automated data, the best performance being obtained with Clinistix. The semiquantitative Clinitest tablets generally yielded more accurate results on a direct urine test than did Tes-Tape, although the Clinitest tablet is designed to measure total reducing substances rather than glucose alone.

SOMMAIRE

Les papiers commerciaux pour l'épreuve de la glycosurie, Tes-Tape, Clinistix, Uristix et Combistix et le comprimé Clinitest ont été évalués comme indicateurs du glucose dans l'urine. On s'est servi à cette fin d'une méthode quantitative automatisée de glucose oxydase pour déterminer le glucose urinaire. Le Tes-Tape semi-quantitatif a donné des chiffres urinaires très faibles par comparaison à la méthode quantitative. On peut obtenir de ce produit des résultats plus précis si l'on prend soin de traiter d'abord le spécimen avec un mélange de résines pour éliminer les inhibiteurs du système d'enzyme oxydase-peroxydase du glucose. Les papiers pour l'analyse qualitative Clinistix, Uristix et Combistix ont donné des réactions très proches des chiffres fournis par la méthode automatisée, le meilleur étant le Clinistix. Les comprimés Clinitest pour l'analyse semi-quantitative ont généralement donné des résultats plus précis sur un échantillon d'urine direct que le Tes-Tape, mais il faut se rappeler que le comprimé Clinitest a été conçu pour mesurer davantage les corps réducteurs que seulement le glucose.

A NUMBER of commercial reagent paper strips and tablets have been used in the laboratory for the past few years for the rapid detection of glucose in urine. Earlier preparations contained reagents to measure the reducing substances in urine and thus were not specific for glucose. Later products have utilized the enzyme reagent system of glucose oxidase peroxidase and are sensitive only to glucose. Since urine contains inhibitors of the glucose oxidase peroxidase system,[1-7] we were interested to determine whether commercial test materials utilizing this principle could yield reliable data when used in the manner indicated by the manufacturer. All values obtained with these products were compared with quantitative data determined using the automated method described by Logan and Haight[1] in which procedure a treatment of the urine by LaMotte mixed bed resin was employed to remove the inhibitors of this enzyme system. In addition to data obtained with these reagent strips, values have also been determined using Clinitest* tablets.

METHODS

Urine specimens for this study were obtained from the Ottawa Civic Hospital Laboratory and contained as preservative the tablets used by the Metropolitan Life Insurance Company.† It was found that these tablets did not interfere in the glucose oxidase peroxidase determination of glucose.[1] The urines were examined using the Clinitest tablets, which measure total reducing substances, and by the reagent paper strips, Clinistix,* Uristix,* Combistix* and Tes-Tape‡. The Clinitest tablets and the Tes-Tape reagent paper were designed to provide semiquantitative results, whereas the other paper strips were prepared to yield qualitative results only. Unless indicated otherwise, the urines were tested without prior treatment and the manufacturers' instructions were followed closely. Following an approximation of the glucose concentration by Tes-Tape, urine specimens containing more than 500 mg./100 ml. glucose were diluted

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*Product of Ames Co. of Canada, Toronto, Ont.

†Manufactured by R. P. Cargille Laboratories, Inc., 117 Liberty Street, New York 6, N.Y.
‡Product of Eli Lilly & Co., Indianapolis, Ind.

Canad. Med. Ass. J.
Sept. 12, 1964, vol. 91

appropriately to yield solutions below this concentration. Approximately 6 g. of LaMotte mixed bed resin was added to 10 ml. of each specimen and the filtrate, recovered after resin separation, was used for automated glucose determination as previously described.[1]

An experiment was carried out to ascertain the differences in semiquantitative data between Clinitest tablets and Tes-Tape reagent paper in determining glucose added to water and to normal urine specimens. In all cases in which weighed amounts of glucose were added to urine or to water, at least two hours were allowed for mutarotation of the glucose to take place before use.

The response of urine to Tes-Tape and Clinistix papers before and after resin treatment was also determined.

RESULTS

Table I compares the values as determined by Tes-Tape with those found using the automated method. There is a very marked underestimation of the amount of glucose when using this reagent paper, compared to our quantitative data. For purposes of evaluation of the semiquantitative readings of Tes-Tape and Clinitest shown in this report, the reading has been designated "confirmed" if the corresponding value obtained on the specimen by the AutoAnalyzer comes within the limits of an arbitrary range assigned to the colour responses of the manufacturers' scales (e.g. a Tes-Tape reading of two plus would be confirmed by any AutoAnalyzer value from 175 to 374). Only 12 out of a total of 84 specimens in the concentration range up to and including 0.5 g. % gave readings by Tes-Tape which checked with the AutoAnalyzer results. In every case of disagreement, the Tes-Tape yielded values lower than the automated method. When the mean values determined quantitatively were compared with the designated Tes-Tape reading of the groups it was found that only 17.0 to 27.8% of the actual amount of glucose was being recorded in the range from 500 mg. % to 100 mg. % The correlation with four-plus responses or 2 g. % or over was much better and in only two cases out of 24 were there unconfirmed readings, viz. overestimation.

Table II shows the comparison of values obtained using Clinitest tablets and the AutoAnalyzer procedure. Despite the fact that these tablets measure total reducing substances, there is better correlation with the quantitative data than there was in the case of Tes-Tape. Up to 750 mg. % there is a tendency for the tablets to give higher readings. However, in the case of the three-plus readings or 1 g. % range, the tablets yielded lower values than those found by the AutoAnalyzer.

TABLE I.—COMPARISON OF TES-TAPE READINGS WITH AUTOANALYZER (AA) VALUES

Tes-Tape reading (mg. %)		*Arbitrary range (mg. %)*	*Mean AA value and range (mg. %)*	*Number of readings confirmed by AA*
100	(+).....	30—174	360 (31—1534)	8/37
250	(++).....	175—374	1322 (176—4801)	2/38
500	(+++).....	375—1249	2943 (1031—5201)	2/9
2000 or over	(++++).....	1250 or over	2941 (845—5112)	22/24
				Total: 34/108

Test results with other commercial papers, Clinistix, Uristix and Combistix, were also compared with data obtained by the automated method; the results are shown in Table III. With these reagent paper strips the manufacturer states, "Usually a light colour corresponds to a small amount (less than ½%) of glucose and a dark colour to a larger amount (more than ½%)." The light and dark responses have been compared with quantitative data, and our results indicate that the best correlation was obtained with Clinistix, especially the "new, improved" product. With Combistix and Uristix, there is a tendency to underestimate the amount of glucose present. Fifteen out of 17 Combistix values and 21 out of 26 Uristix values not correlating were low compared with the automated values.

Table IV shows the result of testing aqueous and normal urine solutions of glucose with Clinitest, Tes-Tape, Clinistix, Combistix and Uristix in order to check the reliability of the calibration of the colour charts provided by the manufacturer. In this table and in Table V colour responses obtained

TABLE II.—COMPARISON OF CLINITEST READINGS WITH AUTOANALYZER (AA) VALUES

Clinitest reading (mg. %)		*Arbitrary range (mg. %)*	*Mean AA value and range (mg. %)*	*Number of readings confirmed by AA*
250	(trace)	50—374	178 (27—441)	12/19
500	(+).....	375—624	429 (22—1380)	4/22
750	(++).....	625—874	657 (24—1534)	8/27
1000	(+++).....	875—1499	1688 (25—3912)	4/14
2000 or over	(++++).....	1500 or over	3120 (28—5779)	28/34
				Total: 56/116

TABLE III.—COMPARISON OF RESPONSES BY QUALITATIVE COMMERCIAL PAPERS WITH AUTOANALYZER (AA) VALUES

	No. correlating with AA value		*Mean AA value and range (mg. %)*	
Name	*Light*	*Dark*	*Light*	*Dark*
Clinistix (old)	8/10	21/30	199 (33—693)	1399 (299—5423)
Clinistix (new)	4/4	22/24	244 (96—363)	2640 (489—5779)
Combistix	14/29	30/32	920 (33—4223)	2672 (176—5779)
Uristix	18/38	32/38	912 (31—4223)	2390 (176—5201)

with Clinitest and Tes-Tape which, in the opinion of the experimenters were intermediate between two of the manufacturer's assigned values, were designated as being greater than (>) or less than (<) than value, depending upon which colour was the more closely approximated. The amount of glucose in the aqueous solutions was overestimated at each concentration by Clinitest but was more closely approximated when the same concentrations were present in urine. With Tes-Tape the sively shorter times for increasingly larger concentrations. In the urine solutions the Clinistix tended to overestimate while the Combistix and Uristix gave closer approximations.

Table V shows the readings obtained on normal urine solutions containing varying amounts of glucose with Clinitest, Tes-Tape and the "new, improved" Clinistix before and after LaMotte resin treatment of the specimen. On the direct urine tests, the Tes-Tape once more yielded low values

TABLE IV.—RESPONSES OF TEST PAPERS AND TABLETS IN AQUEOUS AND URINE SOLUTIONS CONTAINING KNOWN AMOUNTS OF GLUCOSE

Actual glucose conc. mg./100 ml.	*Clinitest*	*Tes-Tape*	*Old Clinistix*	*Combistix*	*Uristix*
H_2O solutions					
100	> 250	100	D (dark)	L (light)	D
250	< 750	250	D	M (medium)	D
500	1000	500	D	D	D
750	>1000	<2000	D	D	D
1000	>1000	>2000	D	D	D
2000	>2000	>2000	D	D	D
Urine solutions					
100	0	< 100	M	Negative	L
250	< 500	100	D	L	M
500	750	< 250	D	M	M
750	750	sl. < 250	D	M	D
1000	>1000	250	D	M and D	D
2000	>2000	2000	D	D	—

aqueous solutions were estimated very well, but in the ranges of concentrations in urine of 1 g. % and lower, the underestimation was marked. Only the 2 g. % solution was correctly estimated. With the qualitative papers, both Clinistix (older preparation) and Uristix overestimated the glucose concentrations in the aqueous solutions, whereas the Combistix approximated the actual value more closely. In the case of aqueous solutions in which a dark response with the qualitative papers was obtained in 10 seconds for a low concentration of glucose, the dark responses appeared in succes- whereas the Clinitest and Clinistix gave approximately correct colour changes. Following resin treatment the Tes-Tape results were more nearly correct whereas the Clinistix responses were too dark and the Clinitest readings were high. It will be noted that a varied response was obtained with the Clinistix strips, particularly on the specimens which had received the resin treatment. The colour changes at the end of 10 seconds were spotty and may be due to insufficient wetting of the strip in this time interval. The removal of salts by the resin may have been a contributing factor. It has, how-

TABLE V.—EFFECT OF RESIN TREATMENT OF URINARY GLUCOSE SOLUTIONS ON RESPONSES BY CLINITEST, TES-TAPE AND CLINISTIX

Actual glucose conc. mg./100 ml.	*Clinitest*		*Tes-Tape*		*Clinistix (new)*	
	*Before RT**	*After RT*	*Before RT*	*After RT*	*Before RT*	*After RT*
100	almost 250	> 250	almost 100	< 100	L (light)	L, M and D
250	250	> 500	> 100	< 250	L and M	M and D
500	sl. > 500	sl. > 750	almost 250	sl. > 250	M (medium)	M and D
750	750	almost 1000	> 250	almost 500	D (dark)	M and D
1000	1000	1000	sl. > 250	almost 500	D	M and D
2000	2000	>2000	2000	sl. >2000	D	D

*RT = resin treatment using LaMotte mixed bed resin.

Canad. Med. Ass. J.
Sept. 12, 1964, vol. 91

ever, been observed during the examination of the hospital urine specimens that there were apparent differences in the wetting property of some of these specimens. The sensitivity of Tes-Tape and Clinistix was compared on aqueous glucose solutions. It was found that first evidence of colour change could be observed with Tes-Tape at 10 mg. % and with Clinistix at 15 mg. %.

DISCUSSION

A number of reports have appeared in the literature assessing the reliability of commercial test papers and tablets for rapid estimation of glucose in urine.[8-18] Considerable difference of opinion as to the usefulness of these products is evident, perhaps owing to the variety of methods used to assess them. Certain conclusions concerning these products may be drawn from our data. Our investigation of the glucose oxidase peroxidase enzyme system confirms the findings reported in the recent literature that uric acid at concentrations found in urine is a potent inhibitor of this enzyme system. From the data presented above, we have shown that resin treatment of the urine specimen for removal of uric acid markedly increases the response obtained with a test paper impregnated with glucose oxidase peroxidase reagents. The question of reliability of results would, therefore, appear to depend upon the calibration of the colour chart provided by the manufacturer. Has the calibration been carried out simply by means of aqueous solutions of glucose of varying concentration or by means of urine plus glucose or aqueous solutions containing the inhibitory concentrations of uric acid normally found in urine? Our data would seem to indicate that the one-, two- and three-plus responses on the Tes-Tape colour chart were incorrectly calibrated, whereas the manufacturer of the qualitative papers, Clinistix, Combistix and Uristix, has attempted to offset to some degree this inhibition effect in the design of a colour scale. The decision to avoid semiquantitation on such a direct urine test is probably a good one.[19] Since the amount of uric acid excreted in urine may vary widely depending upon diet (0.2 g. to over 1 g. daily), it would be difficult to design a colour scale to give the correct readings for glucose in the presence of such variable amounts of enzyme inhibitor. Another factor contributing to this variability would be the wide range of dilution encountered in random urine specimens. For precise results, it would seem best to first treat the urine specimen with ion-exchange media to remove inhibitors and then determine glucose with a paper whose colour scale is calibrated to a set of aqueous standards. Following resin treatment, Tes-Tape, we found, yielded much more reliable data in the range from 100 to 400 mg. %.

Even though Clinitest measured total reducing substances, the data yielded by Clinitest were more often valid as a semiquantitative estimation of glucose in urine than those found with Tes-Tape. The latter, designed to measure glucose specifically, apparently had not been calibrated to offset the effect of glucose oxidase peroxidase inhibitors in urine.

During our study, a "new, improved" Clinistix reagent strip was introduced using a colour scale similar to that of Combistix and Uristix. The older preparation, which used orthotolidine as chromogen, appeared slightly oversensitive in our experiments, and the data obtained did not correlate quite as well with the automated results as did those obtained with the new product.

Jablokow, Hutchins and Knights[15] evaluated Clinistix and Tes-Tape by comparing values obtained with these papers on negative urines to which known concentrations of glucose had been added. With Tes-Tape these workers obtained a higher percentage of reliable results at the one-plus response level but found, as we did, that at the two-plus and three-plus levels the glucose was being consistently underestimated; on the other hand, we obtained much better results than they did at the four-plus level. It is probable that some changes may have been made in the product since the time of their evaluation. With Clinistix, Jablokow, Hutchins and Knights[15] found only five out of 72 urine specimens containing 0.1 g. % glucose that gave a negative test. At higher concentrations, no negative tests were obtained with this product. The conclusions we have drawn from our studies would agree with theirs that these two products are useful qualitative tests for glucose.

Eden[8] and Mann[9] have compared results using Tes-Tape and Clinistix with the Benedict's and Clinitest methods. Both report a reasonable correlation with these two semiquantitative methods which are dependent upon the total reducing substances in the urine. An examination of our data reveals that in nearly every case Tes-Tape indicates a lower concentration range than Clinitest, particularly with specimens containing less than 1 g. % glucose. It is, however, difficult to make an assessment of reagent papers impregnated with the glucose oxidase peroxidase enzyme system by means of methods which measure total reducing substances. In the semiquantitative Benedict's test there appears to be a lack of a standard set of values for interpreting the trace to four-plus responses. In the comparison of Clinitest and Tes-Tape reported by Mann[9] the two tests indicated the same concentration (0.25% and 0.5%) in only 37 out of 193 urine specimens. In all but five of those not correlating, Tes-Tape indicated a lower concentration range than Clinitest. These lower values on testing with Tes-Tape in this concentration range would support our findings.

There are a number of reports in the literature in which the semiquantitative results obtained with Tes-Tape have been assessed. Some workers attest

to its accuracy based on determinations of urine containing known amounts of added glucose.[10-13] Seltzer and Loveall[11] found better agreement of Tes-Tape values with those of a glucose oxidase peroxidase method (Glucostat)* than with those of the Nelson[20] method which measures reducing substances. In this report there was no indication of prior removal of enzyme inhibitors before the Glucostat reaction was carried out. If the inhibitors were not removed, one might expect good correlation with Tes-Tape. Several others have confirmed our findings that Tes-Tape is markedly inaccurate, particularly for measuring levels of glucose in urine of less than 1 g. %.[14-18] The reason for this discrepancy in laboratory findings is difficult to establish. Since the inherent concentration of glucose oxidase peroxidase inhibitors may vary within wide limits depending upon the urine specimens used, it is conceivable that this may account for some variation. However, in view of our findings and those of others that urine does contain inhibitors of this enzyme reaction, it is our contention that the reliability of semiquantitative urinary glucose values obtained by measurement in the presence of these inhibitors is questionable. If these values were used as the basis for the adjustment of insulin dosage, this practice of direct glucose determination in urine could have serious consequences.

*Trademark of Worthington Biochemical Corp., Freehold, New Jersey.

SUMMARY

The accuracy of the commercial test papers, Tes-Tape, Clinistix, Uristix and Combistix, and the tablet preparation, Clinitest, in the determination of glucose in urine was assessed by a quantitative automated procedure using glucose oxidase. Tes-Tape yielded low results on untreated urine specimens but when the urine was first treated to remove inhibitors of the glucose oxidase peroxidase system the results were more reliable. In general, Clinitest gave more accurate estimations of glucose on untreated urine specimens even though the test measures total reducing substances. Of the three qualitative papers, Clinistix yielded results which most closely agreed with the automated data.

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

THE WAR

It is a bitter commentary on our civilization that out of a clear sky such a storm of irrational havoc should suddenly burst on the world. One nation recklessly undertakes to chastise a small but irritating neighbour, and in less than a week all the great nations of Europe are involved in such a war as the world has never seen and, for the credit of humanity be it hoped, never may see again. What a great statesman not long ago described as unthinkable has become a reality, the full horror of which cannot yet be realized. Still, it is perhaps only the inevitable result of the policy of blood and iron by which the German states were welded together, and which has imposed upon Europe an increasingly wasteful burden of armaments. This, however, is not the place to discuss the political motives of the protagonists. It is enough for us that the Empire which is our heritage, is involved in a struggle for self-preservation, and on the side of the weak against the strong, of international justice against brutalizing militarism. Meanwhile the first great victory has been won by the British fleet. Within a week of the outbreak of hostilities, the safety of the paths of the sea, upon which the existence of the Empire depends, was practically assured. If only all the victories of this war could be so swift, so decisive, and so bloodless!

The vast numbers of the opposing armies and the deadly efficiency of the modern engines of destruction make it certain that the sacrifice of life and limb will be appalling. But with the progress of preventive medicine the military medical services have acquired a vastly increased importance, and it is reasonable to expect that disease, which in the past has been a more deadly enemy to the soldier than the bullet, will now play a minor rôle. Typhoid, particularly, should be prevented from repeating the ravages it made in South Africa and in the Spanish American War, in both of which campaigns it claimed by far the larger share of victims.

In England factional strife is forgotten, and the crisis has been met in an admirable spirit and with a grim realization of the magnitude of the task which has to be performed. Canadians, too, are all of one heart and mind, anxious to do all that they can, and ready to make whatever sacrifices the fortune of war may demand. And it is a matter of congratulation that our profession is perhaps better prepared for the emergency than any other class in the community. In the first Canadian Division, numbering over twenty thousand men, which is now being mobilized at Valcartier, will be included fifty medical officers and over seven hundred non-commissioned officers and men, constituting the medical corps. There has been no difficulty in recruiting the field ambulances up to war strength, and in filling the ranks with a fine class of men, and divisional headquarters in the larger cities have been flooded with offers for service from both doctors and nurses.—Editorial, *Canad. Med. Ass. J.*, 4: 803, 1914.